THE HEMORRHAGIC SYNDROME OF ACUTE IONIZING RADIATION ILLNESS PRODUCED IN GOATS AND SWINE BY EXPOSURE TO THE ATOMIC BOMB AT BIKINI, 1946

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THE PROMINENCE and importance of the hemorrhagic syndrome of acute radiation illness have been amply demonstrated.[1-4]

Shouse, Warren and Whipple[4] described a severe intoxication in dogs produced by x-ray irradiation. Hemorrhage into all tissues was emphasized as an important factor causing death. This hemorrhagic syndrome was assumed to be the result of a rapid disappearance of platelets during the last few days of life. Dogs survived if a small area of the skeleton was shielded from radiation. This suggested to the authors that platelet production in the shielded area prevented the severe hemorrhage. However, they believed that other unknown factors may also have been concerned in the pathogenesis of the acute hemorrhage in the last day of life.

Following the atomic bomb explosions at Hiroshima and Nagasaki, a severe hemorrhagic syndrome appeared in many casualties.[5-10] Warren[5] observed whole blood coagulation times as long as four hours. In addition there was a marked reduction in circulating platelets and a depletion of megakaryocytes from the bone marrow. The above reports on human atomic bomb casualties emphasize the thrombocytopenia and in general tend to ascribe the appearance of the clotting defect to the thrombocytopenia which was usually present in the Japanese when the hemorrhagic phase of acute radiation illness appeared. Increased capillary fragility was also observed in the casualties.

Allen et al.[11, 12] demonstrated a circulating anticoagulant with heparin-like properties in dogs after exposure to 450 r total body x-ray irradiation. The anticoagulant was neutralized by known antiheparin agents.[13-16]

This preliminary paper based on the field tests at Bikini in July 1946 is being reported because of the public and scientific interest in the atomic bomb tests. In addition, the data emphasize certain factors concerned with the hemorrhagic syndrome of radiation illness that are not widely appreciated at this time. It is fully recognized that the work was performed under field conditions and that in many respects it is incomplete.

Materials and Methods

Of 176 goats exposed during the aerial atomic bomb explosion 31 developed detectable effects of radiation in one form or another. Of these 31 goats, 14 developed marked purpura and 4 developed a prolonged clotting time. The goats with purpura and particularly those with a blood coagulation defect will be considered in respect to the hemorrhagic tendency. Methods of exposure and pathologic and clinical descriptions of all the animals exposed have been previously reported.[18-21]

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For the underwater atomic bomb explosion a total of 20 swine in two groups of 10 each were placed aboard two target ships. All animals were below decks, protected from air blast and thermal injuries. The ships were closed, as at general quarters, so that direct contamination with radioactive materials would be unlikely unless the integrity of the ship was broken. It is considered that all pathologic phenomena observed were the result of exposure to gamma radiation either at the time of the explosion or from continued gamma emission from fission products showered upon the ships after the explosion. The greater part of this radiation was received during the first two days. Solid blast injuries were a possibility but there were few clinical or pathologic findings which could be attributed to this type of injury.

Prior to the tests control blood studies were performed on the goats and swine. Normal values for red blood cell counts, hemoglobin determinations, hematocrit readings, white and differential blood cell counts, platelet counts, prothrombin and whole blood clotting times, and clot retraction times were established. After the tests when the animals were returned to the laboratory ship, these studies were continued. When time was a limiting factor, samples of plasma were frozen for future study. Methods of performing the coagulation studies will be reported elsewhere.[22]

Results

Goats

On July 1, 1946, the aerial atomic bomb was detonated. Goats and swine received doses of radiation varying from an insignificant amount to over 10,000 r in a very short period of time. Due to the bombing error very few animals were exposed to a midlethal dose of radiation. Eight goats received very high absolutely lethal doses, much more than 1000 r. These animals lived for only a few days (less than seven), and did not develop visible purpura. No treatment was given. At autopsy, scattered petechiae were seen. Observations on blood coagulation were not made in these goats. Platelets were present in large numbers on the blood smears of all of these goats. Clotted blood was found in the blood vessels at autopsy performed immediately after death.

Eight goats received in the vicinity of 1000 r. These animals lived between nine and 15 days after the explosion. Marked purpura developed between the seventh and twelfth days after exposure. All died by the fifteenth day, and very extensive purpura with clotted blood in the vessels was found at autopsy performed immediately after death. Coagulation studies were performed on all of these goats beginning on the fifth day after the explosion: On the fifth, sixth and seventh days no significant abnormalities of the coagulation mechanism were found. From the ninth to the sixteenth days after exposure there was an occasional mild increase in the blood coagulation time. Infrequently the one stage prothrombin time was increased for no obvious reason (table 1). Goats Nos. 150, 108, 57 and 49 developed a definite defect in blood coagulation at some time before death. Table 2 contains the special studies. Clot retraction was uniformly poor or failed to occur after platelets* became scarce on the blood smears.

Lysis of the clots at the end of twenty-four hours was either partial or complete. In some instances, toluidine blue in vitro returned the blood clotting and prothrombin times to normal. In every case, thrombin clotted the plasma at the same or a more rapid rate than occurred in the one stage prothrombin system. The addition of thromboplastin to whole blood decreased the blood coagulation time

* Platelet counts in goats were too inaccurate aboard ship to be reported.

TABLE 1.—*Whole Blood Coagulation Time and Prothrombin Time of Goats Receiving in the Vicinity of 1000 r Atomic Bomb Radiation*

Goat No.	Days after exposure									
	5	6	7	9	10	11	12	13	14	15
150 CT	10′	9′	12′		14′	IC*	28′	39′	20′	65′
PT	13″	13″	10″		14″	16″	12″	12″	12″	10″
111 CT	10′	7′			Died					
PT	11″	14″								
108 CT				19′	7′	20′		Died		
PT				15″	15″	14″				
57 CT			12′		20′	9′	21′	Died		
PT			19″		19″	15″	15″			
54 CT			13′		13′		15′	Died		
PT					15″					
60 CT										
PT										
49 CT	8′				IC*	Died				
PT	18″				45″					
52 CT		9′								
PT		13″				Died				

CT, Clotting time of whole blood. Normal mean 8.8 min. Range 4–15 min.
PT, One stage prothrombin time. Normal mean 13.2 sec. Range 9–18 sec.
IC, Incoagulable.
* After transfusion of 500 cc. coagulation time was 25′.

TABLE 2.—*Blood Studies that Relate to the Hemorrhagic Syndrome in Goats 150, 108, 57 and 49 between the 9th and 16th Days after Exposure. All Cases Fatal.*

Days after exposure	Goat No.	Blood Clotting time in Minutes	One step Prothrombin time in sec.	Clot retraction after 24 hr.	Clot lysis after 24 hr.	Amount of toluidine blue in gamma per ml. to return clotting time to normal		Plasma clotting time with thrombin in sec.*	Thromboplastin concentration that reduced the blood clotting time to less than 1.5 min.
						Blood	One stage prothrombin time		
9	108	19	15	Poor	Partial	†	†	10	10^{-4}
10	57	20	19	Poor	Partial	†	†	8	10^{-5}
10	49	60	45	None	Complete	18	18	12	10^{-1}
11	150	60	16	None	Complete	20	20	16	‡
12	150	28	12	None	Complete	—	—	10	10^{-1}
13	150	39	12	None	Complete	4	4	10	10^{-4}
14	150	20	12	None	Complete	—	—	8	10^{-5}
15	150	65	10	None	Complete	—	—	8	10^{-1}

* Clotting time in seconds with pure Lederle hemostatic globulin.
† Did not return time to normal.
‡ Undiluted thromboplastin reduced clotting time to 5 minutes.

of each sample tested. Scattered blood transfusions were administered to these animals and penicillin was given during the last few days of life. Details have been previously reported.[21]

Six goats received more than 100 r but less than 1000 r. More precise measurements on the amount of radiation received have not been made available. It is only reasonable to assume that the exposure was less than 300 r because none of these animals died during the first 30 days after exposure. However, these goats were treated intensively with blood transfusions and penicillin which may have prolonged their lives and altered the course.[21] All developed purpura when platelets became scarce on the blood smears. Between the fifth and twenty-fifth days after exposure the blood coagulation was followed. The clotting time remained normal. Visible purpura had disappeared by the twenty-first day and on the twenty-fifth day, studies on the coagulation mechanism were discontinued.

Nine goats received less than 100 r. None of these died. There was no purpura and there was no alteration in the coagulation mechanism.

Swine

The results of hematologic studies on the swine exposed at Bikini during the underwater test are reported in detail. These swine can be divided into two groups on the basis of their pooled leukocyte counts, exposure to radiation, and clinical picture. Group A received approximately 20,000 r over a period of five days. Only 4 of the 10 animals placed on this ship were alive when the ship was boarded five days after the explosion. Group B consisted of 10 swine: 5 that received about 1500 r over a period of five days, 5 that received about 1300 r over a period of four days.

Group A

The 4 survivors from Group A (20,000 r) were active upon recovery. Diarrhea was present in all. A few streaks of bloody mucus were seen in the stools. A few scattered petechiae were seen in the skin, and there was definitely an increased tendency to bleed from needle punctures. Respiratory rate was increased. The animals were not eating or drinking. Though there were no changes in the red blood counts, hemoglobin or hematocrit readings, the white blood cell counts were extremely low. The average total white count was 700 per cu.mm. and the average lymphocyte count was 470 per cu.mm. (table 3).

Capillary fragility was not tested in the Group A swine. The lowest platelet count was 100,000/cu.mm. The average clotting time was slightly prolonged from 5.5 minutes to 8.5 minutes, which is still within the normal range for swine. The average prothrombin time was inexplicably prolonged from 10 seconds to 16 seconds (table 3). The clots retracted slowly but retraction was complete in twelve hours. Clots dissolved after twenty-four hours of refrigeration. Cultures were not performed on these clots. Toluidine blue titrations were not performed on the blood of this group. Temperatures were not taken. All animals died on the night of the fifth day after the explosion.

Autopsy findings are reported separately.[18, 19] However, in general it can be said that the bowel was badly injured throughout and covered in large part with a greenish membrane. There was a moderate degree of purpura of all organs.

Group B

Group B swine presented a more varied clinical picture. After return from the target ships these animals were eating and drinking, and were normally hyperactive. Stools were normal or soft. The rectal temperatures were in the normal range (100.5–102.5 C.). On the sixth to eighth days after exposure, these animals became listless and ate poorly. Small petechiae began to appear in the conjunctivae and on the oral mucous membranes on the eighth or ninth day. Cutaneous purpura became marked on the tenth day. Diarrhea appeared on the sixth day and became increasingly bloody from the eighth day until death. Melena was prominent the last day of life. From the sixth day until death, rectal temperatures progressively

TABLE 3.—*Hematologic Data, Group A Pigs*

Pig. No.	Total, WBC × 10^3 /mm.		Total, Lymph × 10^3 /mm.^{3}		Total, RBC × 10^6/mm.^{3}		Hemoglobin, Gm./100 cc.	
	Control	5 days after exposure	Control	5 days after exposure	Control	5 days after exposure	Control	5 days after exposure
459		0.4		0.3		5.6		16.3
419	25.0	0.7	20.2	0.0	5.5	6.2	9.0	14.6
364	27.2	1.5	15.5	1.35	5.4	4.8	13.5	12.7
265	27.0	0.3	18.9	0.245	6.7	6.1	14.6	15.8
Average	26.4	0.7	18.2	0.47	5.8	5.5	12.3	14.8

Pig No.	Hematocrit, %		Platelets, × 10^3 /mm.^{3}		Clotting Time, Minutes		Prothrombin, Time in Seconds	
	Control	5 days after exposure	Control	5 days after exposure	Control	5 days after exposure	Control	5 days after exposure
459		42		180		8		16
419	39	42		200	6	7	11	15
364	42	35	310	170		12		15
265	36	42	500	280	5	7	9	18
Average	39	40	405	207	5.5	8.5	10	16

increased (table 4). Terminally, there was severe, extensive hemorrhage into the skin and the mucous membranes with a continuous trickle of blood from the nose, the vagina, rectum and urethra of all animals.

Red blood cell count, hemoglobin, hematocrit, red corpuscle indices. These determinations in Group B decreased slowly at first and then increased slightly with the onset of the diarrhea and failure to eat and drink. When the bleeding began there was a progressive drop in the red count, hemoglobin, and hematocrit values. Corpuscular indices remained constant. There was no gross evidence of hemolysis. Serum bilirubins were not done; however, the plasma remained clear. Changes in the erythron of these animals seemed largely due to hemorrhage and the cessation of blood for-

mation, but the possibility of increased hemolysis was not eliminated by chemical studies and may have contributed in part.

White and differential blood cell counts. The changes in the Group B average total white cell count are demonstrated in figure 1. On the Wright stained blood smears

TABLE 4.—*Swine Rectal Temperatures, Underwater Test, Group B*

Animal No.	Days After Exposure					
	4	5	6	10	11	12
342	103^{6}		104	106^{8}		
272	102		102^{6}	102		
344	103^{2}		103	105^{2}		
330	102^{6}		104	107	107	105^{8}
337	102		102^{6}	107	107	107
456	102^{8}		102^{4}	106^{2}	106^{2}	106
393	102		104	106^{8}		
420	103		103^{8}	107^{8}	107^{6}	
455	103^{4}		102^{6}	106		
Average	102^{6}		103^{2}	106^{2}	106^{9}	106^{3}

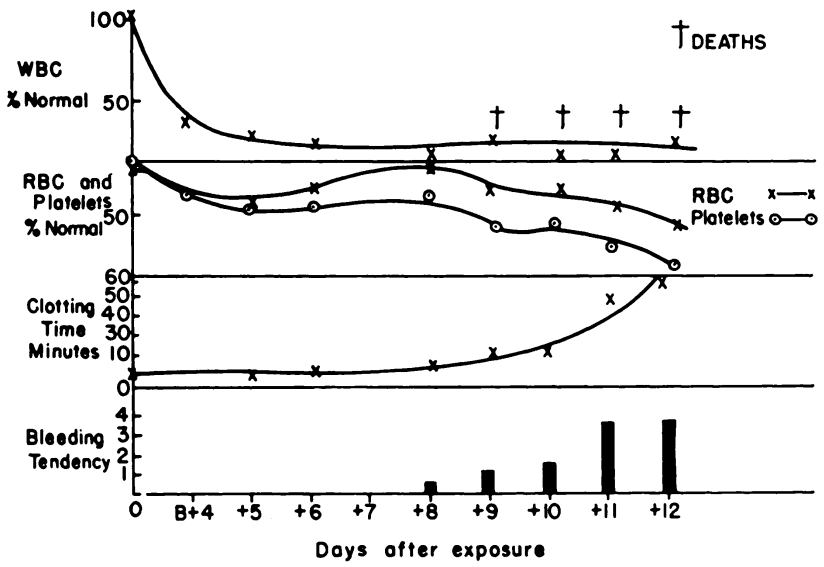

FIG. 1.—Composite graph showing the relationship of the cellular elements and clotting time to the development of the hemorrhagic state. Average daily values are plotted for Group B.

the percentage of degenerating cells was high and most of the white cells were abnormal in appearance. The cytoplasm was vacuolated and unusually basophilic. Heterophiles* of the pig normally have very small, almost invisible granules. A few cells were seen that had large basophilic granules. Nuclear patterns were

* Heterophiles used as defined by Bloom.[35]

bizarre or disrupted, and occasional mononuclear cells were seen resembling those of infectious mononucleosis of man. The immature nucleated red cells seen in goats and swine after the air explosion[21] were absent in these animals. Bacteria were frequently seen in the blood smears after the ninth day.

Platelets. Platelet counts at best are approximate. Aboard ship the inaccuracy is increased. Platelets were rarely seen on the stained smears after the ninth day. This suggests that the platelet counts are in error on the high side. However, there was a steady trend downwards at a less rapid rate than the leukocytes. Of the platelets seen, some were abnormally large and had hyperchromatic centers.

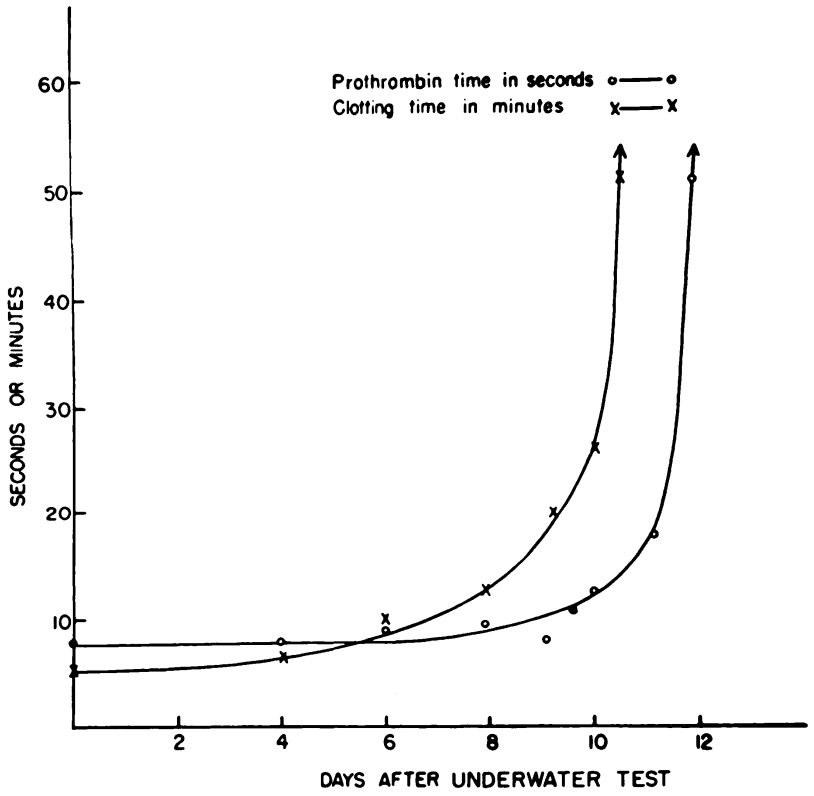

FIG. 2.—Pooled whole blood clotting and prothrombin times for Group B.

The hemorrhagic tendency. Hemorrhage was the most prominent feature in Group B. The tendency to bleed can be divided into three stages. The first stage prior to the eighth day was characterized by a few scattered petechiae at a time when platelets were present in adequate numbers, and there was no significant defect in the clotting of blood; but capillary fragility was increased as indicated by positive tourniquet tests. The second stage occurred on the ninth and tenth days after exposure and was characterized by a definitely increased capillary fragility and a thrombopenia. The third stage was apparently a terminal stage in which there was a marked increase in capillary fragility, a severe thrombopenia, and at times failure of the blood to clot properly, if at all (fig. 1).

Phenomena connected with the clotting defect. The most obvious abnormal finding was

an increase in the whole blood clotting time in Group B beginning between the sixth and the eighth days after exposure. After the tenth day the blood coagulation time of these pigs was prolonged in vitro. Increase in the one stage prothrombin

FIG. 3.—Graphic demonstration of the coagulant action (antiheparin action) and the anticoagulant action of toluidine blue on the whole blood coagulation time of fatally irradiated swine.

TABLE 5.—*Studies Performed on the Blood Coagulation of Swine in Group B on the Day of Death*

Pig No.*	Days After Exposure	CT in Minutes	PT in Seconds	Reduction in Clotting Time With			Addition to Normal Blood Prolonged CT
				Toluidine Blue	Thrombo-plastin	Thrombin	
272	9	8	—	No	Yes	Yes	—
420	10	18	10	"	"	"	—
344	10	18	4	"	"	"	—
393	11	28	13	Yes	"	"	—
342	11	35	20	"	"	"	Yes
456	11	20	10	"	"	"	"
337	12	50	30	"	"	"	"
402	12	58	20	"	"	"	"
330	13	>60	10	"	"	"	"
455	13	57	10	"	"	"	"

* Bikini test number.
CT, Clotting time in minutes, Average 6.6, Range 4 to 10.
PT, Prothrombin time in seconds, Average 10.8, Range 7 to 15.

time lagged behind the whole blood clotting time (fig. 2). Using a method modified after Allen,[17] it was possible to reduce the clotting time on the eleventh, and twelfth days after exposure by adding toluidine blue (fig. 3). Similar observations were made on the frozen plasma of 6 pigs in Group B at a later date, utilizing a

one-stage prothrombin system. In all cases the whole blood coagulation time was increased and the addition of 0.1 cc. of rabbit thrombin* to 1 cc. of the pig blood resulted in a fragile clot within three minutes. However, the clots did not retract well and they dissolved within twenty-four hours. The addition of thromboplastin in large amounts accelerated the coagulation to a great extent and in some cases brought the coagulation time down to normal range. When equal parts of freshly drawn normal pig blood were added to the incoagulable blood, the coagulation time was reduced to a certain extent but not returned to the normal range; i.e., the mixture of equal parts of blood that had a clotting time of 60 minutes with normal blood that had a clotting time of 10 minutes resulted in a clotting time of 45 minutes (table 5).

Discussion

Before discussing the hemorrhagic syndrome of radiation illness per se it seems appropriate to consider briefly the natural defenses against hemorrhage. The first and most obvious defense is the integrity of the vascular system itself. Before hemorrhage can take place there must be breaks in the walls of the vascular bed. When a break occurs, a series of events take place that normally will quickly stop the loss of blood. First, there is a spasm of the regional and injured vessels that reduces the volume of blood to the area. Second, platelets adhere to the traumatized surfaces, and in the case of capillaries and small veins where the pressure is low the holes are satisfactorily plugged by a platelet mass. Third, fibrin formation is initiated by the release of thromboplastic substances from the injured tissue. Fibrin formation is of importance in hemostasis, particularly on the arterial side of the vascular system. This can be inferred phylogenetically, inasmuch as fibrinogen does not make its appearance until a high systolic pressure is necessary for the organism. In addition, the bleeding of hemophiliacs is predominantly from traumatized vessels on the arterial side and not from the capilllary bed. Similarly, the excessive dosage of heparin in man or animals results in a hemophilic-like state rather than a purpuric state. Hence the necessary prerequisite for a spontaneous hemorrhagic syndrome is loss of the integrity of the vascular bed. If this is accompanied by vasodilatation, a thrombopenia, or a coagulation defect, the control of hemorrhage will be difficult, and in the case of failure of the blood to clot, bleeding from the arterial side may become spontaneously uncontrollable.

In considering the hemorrhagic syndrome of radiation illness, the factors that cause the loss of integrity of the vascular bed are of primary importance. There are two factors to be considered: (1) There is a widespread increase in vascular fragility to an extent where the traumata of existence disrupts small vessels. From the available literature[1-12] and from the Bikini studies[18, 19] it can be presumed that the increased fragility is a result of either the direct or indirect effects of radiation on the endothelium and its supporting structures aided by "intoxication" and infection. Vascular disintegration has been emphasized by Field and Rekers.[34] Mild purpura without a thrombopenia or clotting defect was observed in the Bikini animals that received massive doses of radiation and lived only a few days. It is

* Lederle Hemostatic Globulin. With this sample of thrombin the concentration was not known.

assumed that the "increased capillary fragility" is due to some injurious process. It is noteworthy that morphologic evidence of damage to the endothelium and its supporting structure during the early phase (first fifteen days) has not been presented. Bloom and associates[35] on a purely morphologic basis have emphasized late radiation injury to the supporting structures of small blood vessels. (2) There is erosion into larger vessels by ulcerative processes of the skin and mucous membranes. These ulcerations are in general secondary to the profound granulocytopenia. Hemorrhage from ulcerations was seen frequently at Bikini. For example, massive hemorrhage from ulcerations of the stomach of rats and the renal pelvis of goats and hogs was seen. In these cases, firmly clotted blood was found at autopsies performed immediately after death.[18, 19]

Of course, vasodilatation as seen[18, 19] in the skin and mucous membranes will accentuate the bleeding tendency.

The problem of availability of an active form of thromboplastin in the tissues of irradiated animals has not been adequately studied. Preliminary studies at the Naval Medical Research Institute suggest that tissue thromboplastins are equally as potent in irradiated animals as in normal animals.

The importance of a thrombocytopenia in the presence of a hemorrhagic tendency due to vascular erosions or increased fragility cannot be minimized. Allen and associates[11, 12] did not find a parallelism, however, between the tendency to bleed and the thrombopenia. At Bikini, the tendency to bleed seemed to be accentuated by low platelet levels, but not enough serial studies were performed to show strict parallelism. In the dogs studied by Field and Rekers,[34] the mean platelet counts were 50,000 per cu.mm. or less between the tenth and twenty-third days after irradiation, when hemorrhage was most prevalent. Shouse et al.[4] emphasized a thrombopenia in dogs as did those who studied the Japanese.[5–10] Dixon[3] has demonstrated a severe radiation-induced hemorrhagic syndrome in chickens by the continuous internal radiation with P^{32}. He concluded that a profound thrombocytopenia with resulting poor clots was the main cause. A prolonged clotting time was not found. Subsequent work at this Institute has shown that a reduction in or complete disappearance of platelets is a uniform occurrence in swine, goats, rats, dogs, guinea pigs, and mice that develop a severe and fatal hemorrhagic syndrome. In swine that survived an LD 12.5 per cent* of 1000 KV x-ray, a thrombocytopenia with purpura was observed[27, 32]; the clotting time of whole blood and plasma was not prolonged.

Apparently in all animals that develop a severe hemorrhagic syndrome there is a thrombocytopenia, accompanied by poor clot retraction and a fragile clot.[4–12, 21, 27, 33, 34] Since this type of clotting defect is usually associated with a thrombocytopenia, it is concluded that a vascular defect and thrombocytopenia are the main factors in the pathogenesis of hemorrhagic syndromes. In addition, some species of animals, at least under certain conditions or after certain doses of radiation, develop a clotting defect characterized by a prolonged whole blood coagulation time with evidence suggestive of a "heparinemia." For example, this was demonstrated by Allen and associates[11, 12] for dogs after they had received 450 r

* 200 r in air to both sides.[32]

whole body irradiation and in some of the goats and swine at Bikini. However, a prolonged clotting time in dogs exposed to 350 and 450 r of whole body irradiation was not observed by Field and Rekers,[34] although a severe and frequently fatal hemorrhagic syndrome was produced. All of the goats and swine that we saw, and apparently all of Allen's dogs, that developed a prolonged clotting time died. In the light of our present knowledge a failure in the clotting mechanism seems to indicate a rapidly fatal outcome. The mechanism of development of this increased blood clotting time observed in some Bikini animals is not clear. The prolongation of the blood clotting time, the terminal increase in the one stage prothrombin time and the tendency for clots to undergo lysis are phenomena which suggest the presence of activated fibrinolysins. Activation of the fibrinolytic power of blood and the presence of heparin and histamine have been reported in anaphylactic shock.[25] Unfortunately, direct assays for fibrinolysins were not performed at Bikini and the possibility that the clots were lysed by bacterial growth was not eliminated. The prolongation of the clotting time of normal blood by an equal amount of the blood with a prolonged clotting time suggests that an anticoagulant is present. The reduction in clotting time with thrombin, thromboplastin and toluidine blue suggests that the anticoagulant may be heparin or some related substance. At least heparin will combine with or neutralize to a certain extent all of the aforementioned substances. However, the direct isolation of heparin was not attempted at Bikini. In the light of our present knowledge, based on the original work of Allen[11, 12] and the present report, it is clear that anticoagulants occasionally appear in the blood stream of dogs, swine and goats that have been exposed to varying amounts of total body radiation that have a *lethal* result between about the tenth and twentieth days after irradiation. Is the occasional appearance of this anticoagulant with heparin-like properties due to an increased production and liberation of heparin into the blood stream, to an inability of the organism to dispose of heparin, or is something occurring which greatly increases the effectiveness of the amount of heparin that is normally present?* The evidence for excess production of heparin-like substance and its release from mast calls has been covered by Allen and associates.[11, 12] Hypothetically, the possibility that heparin cannot be disposed of is suggested by the work of Iankovskii,[28] who concluded that the bowel of dogs actively takes up and destroys injected heparin. In the goats and swine that had a prolonged blood coagulation time at Bikini there was extensive necrosis and ulceration of the bowel. However, Jaques et al.[31] have not been able to duplicate the work of Iankovskii. The evidence for increased effectiveness of heparin due to some other factor is scanty. In one irradiated dog, Holden[29] has observed a great increase in the concentration of "heparin co-factor" which probably represents the normal antithrombins that are necessary for the antithrombic activity of heparin. Whether an increased titer of heparin co-factor will increase the effectiveness of trace amounts of heparin is not known, nor are the reactions between co-factor, thromboplastin, toluidine blue, or protamine known. The mechanism of release of the anticoagulant, its precise nature, and the reasons for its inconstant appearance are obscure.

* Heparin has been recently demonstrated in the blood of normal man and dogs.[30]

In the Bikini animals a seeming paradox was also observed. Some of the swine and goats with a coagulation defect so severe that the blood was incoagulable shortly before death presented firmly coagulated blood in the pelvis of each kidney at autopsy, which was performed immediately after death. Microscopically, fibrin was seen in the lungs, intestinal ulcerations and some blood vessels as well as in the obvious blood clots.[19]

The inconstancy with which the blood coagulation time is prolonged in radiation illness has its counterpart in the other conditions which presumably produce an increased blood coagulation time and "heparinemia," e.g., nitrogen mustard poisoning and peptone shock. In a patient treated with large doses of nitrogen mustard for metastatic seminoma of the testicle and in 2 swine given large doses of nitrogen mustard a severe hemorrhagic syndrome was produced while the coagulation time and the heparin tolerance* remained normal. Platelets almost disappeared from the blood. An attempt to produce a prolonged clotting time by peptone shock in 2 swine also failed despite a severe reaction.

In searching for a common denominator for a biologic phenomenon common to many species, such as the hemorrhagic syndrome of radiation illness, it is necessary to demonstrate its presence in all species and throughout the whole dose range in which the given effect is produced. "Heparinemia" cannot, in the light of present knowledge, be considered the most important or universal cause of radiation hemorrhage.

The analysis of radiation illness is complicated by the difficulty in segregating the phenomena that are a direct result of the radiation produced by ionization in the tissues from those phenomena that may be a result of bacterial invasion or reparative processes within the body. Ellinger has emphasized this fact, and in addition, with others, has repeatedly called attention to the fact that radiation phenomena are a function of and vary with the dosage of radiation.[21, 23, 24] In general, the phenomena that were observed in the Bikini animals during the first seventeen days after exposure can be considered due to the sequence of events set in motion by the initial radiation injury. Following the ninth day after exposure, bacterial invasion is observed with increasing frequency. When blood stream infection occurs, the direct effects of radiation are obscured. This fact must be considered in evaluating the delayed effects of irradiation. It is during the period when infections are most prominent that the blood coagulation time is found to be prolonged. It is conceivable that the "heparinemia" when it occurs is related to bacterial invasion and extensive necrosis and intoxication.

It should be emphasized that technics for the isolation and detection of heparin are very crude. However, amounts of heparin too small to be determined after addition to blood will greatly prolong the clotting time. Hence, in the absence of a significantly prolonged clotting time it is difficult to conceive of a "heparinemia." Lastly, clotting times must be performed under very highly controlled laboratory conditions. The temperature must be kept constant because decreases in the temperature below the physiologic range increase the coagulation time. Temperatures above the physiologic range will shorten the coagulation time.

* Protamine heparin tolerance test of Allen.[17]

Summary and Conclusions

1. The hemorrhagic syndrome of acute radiation illness in goats and swine has been described. This syndrome is predominantly a result of a combination of "increased vascular fragility" and thrombopenia. Infrequently, a blood coagulation defect characterized by a prolonged clotting time due to a circulating anticoagulant with heparin-like properties appears, thus confirming under some conditions in goats and swine the work of Allen on "heparinemia" in irradiated dogs.

2. The prolonged blood coagulation time appeared only in fatally irradiated goats and swine.

3. Evidence was presented suggesting that serum fibrinolysins may have been activated.

4. It is concluded on the basis of this work and that of others that a hemorrhagic syndrome can develop in irradiated dogs, goats, swine, rats, chickens and guinea pigs without the appearance of a prolonged clotting time and without a detectible "heparinemia." The biologically most universal phenomena observed in the hemorrhagic syndrome of radiation illness appear to be: (a) increased vascular fragility, (b) thrombopenia, and (c) ulcerations.

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REFERENCES

[1] Fernau, Schramek and Zarzicki: Über die Wirkung von induzierter Radioaktivität. Wien. klin. Wchnschr. *26:* 94, 1913–1925.

[2] Falta: Quoted by Lacassagne and Lavedan.[3]

[3] Lacassagne, S. L., and Lavedan, J.: Etude experimentalle des effects biologiques du polonium introduit des l'organisme. J. de radiol. et d'electrol. *9:* 1–14, 67–82, 1923.

[4] Shouse, S. S., Warren, S. L., and Whipple, G. H.: Aplasia of marrow and fatal intoxication in dogs produced by roentgen radiation of all bones. J. Exper. Med. *53:* 421–435, 1931.

[5] Warren, S.: Notes on observations and studies made on the effects of atomic bomb explosions at Hiroshima and Nagasaki. BuMed News Letter *7:* No. 9, March, 1946.

[6] Larkin, J. C.: The distribution of radiation in the atomic bombing of Nagasaki. Am. J. Roentgenol. *55:* 525–532, 1946.

[7] Warren, S.: Radiation effects of atomic bomb likened to 500 r-x-ray dose. Hospital Management *61:* 138–142, 1946.

[8] ——: Pathologic effects of an instantaneous dose of radiation. Cancer Research *6:* 449–453, 1947.

[9] Beck, J. S. P., and Meissner, W. A.: Radiation effects of the atomic bomb. Am. J. Clin. Path. *16:* 586–592, 1946.

[10] LeRoy, G. W.: The medical sequelae of the atomic bomb explosion. J. A. M. A. *134:* 1143–1148, 1947.

[11] Allen, J. G., Sanderson, M., and Jacobson, L. O.: Hyperheparinemia the cause of the hemorrhagic disease produced by total body exposure to ionizing radiations. MDDC 836, Nov., 1946.

[12] ——, and Jacobson, L. O.: Hyperheparinemia cause of the hemorrhagic syndrome associated with total body exposure to ionizing radiation. Science *105:* 388–389, 1947.

[13] Chargaff, E., and Olson, K. B.: Studies on the chemistry of blood coagulation. VI. Studies on the action of heparin and other anti-coagulants. The influence of protamine on the anticoagulant effect *in vivo*. J. Biol. Chem. *122:* 153–157, 1938.

[14] JAQUES, L. B.: The reaction of heparin with proteins and complex bases. Biochem. J. *37:* 138–195, 1943.
[15] JORPES, J. E.: Heparin in the Treatment of Thromboses, ed. 2. London, Oxford University Press, 1946.
[16] JAQUES, L. B., MITFORD, M. B., AND MACDONALD, A. G.: Unpublished article cited by ROCHA E SILVA, M., ET AL.: Liberation of histamine and heparin from the isolated dog's liver. Proc. Soc. Exper. Biol. & Med. *64:* 141–146, 1947.
[17] ALLEN, J. G.: Personal communication.
[18] TULLIS, J. L., AND WARREN, S.: Gross autopsy observations in the animals exposed at Bikini—a preliminary report. J. A. M. A. *134:* 1155–1158, 1947.
[19] ——: Response of tissue to total body irradiation, Am. J. Path. In press, 1949.
[20] DRAEGER, R. H., AND WARREN, S.: Medicine at the crossroads. U. S. Naval Med. Bull. *47:* 219, 1947.
[21] CRONKITE, E. P.: The clinical manifestations of acute radiation illness produced in goats by exposure to an atomic bomb, Test Able, 1946, with comments on therapy. U. S. Naval Med. Bull. In press, 1949.
[22] ——, AND CHARTERS, W. V.: In preparation.
[23] ELLINGER, F. P.: The Biologic Fundamentals of Radiation Therapy. New York, Elsevier Publishing Co., Inc., 1941.
[24] ——: Lethal dose studies with x-rays. Radiology *44:* 125–142, 1945.
[25] ROCHA E SILVA, M.: Activation of the fibrinolytic power of the blood in anaphylactic and peptone shock. Abstract from Seventeenth International Physiological Congress, 1947, London.
[26] CRONKITE, E. P.: Hematology of the Bikini animals. Abstract, Am. J. Path. *23:* 891, 1947.
[27] ——: Investigation in progress.
[28] IANKOVSKII, V. D.: J. Biol. Med. Exper. USSR *14:* 44, 1930 (Physiol. Abstr. *18:* 324, 1933).
[29] HOLDEN, W. D.: Personal communication.
[30] JAQUES, L. B., MONKHOUSE, F. C., AND STEWART, MARY: A method for the determination of heparin in blood. To be published.
[31] ——, AND RICKER, A. G.: The relationship between heparin dosage and clotting time. Blood *3:* 1197–1212, 1948.
[32] TULLIS, J. L., TESSMER, C. F., CRONKITE, E. P., AND CHAMBERS, F. W., JR.: The lethal dose of total body x-ray irradiation in swine. Radiology. In press, 1949.
[33] DIXON, F. J.: Radiation induced hemorrhagic disease in chickens. Proc. Soc. Exper. Biol. & Med. *68:* 505–507, 1948.
[34] FIELD, J., AND REKERS, P.: Ionizing radiation disease and a study of the protective nature of flavonones. MDDC 1672. U. S. Atomic Energy Commission, 1948.
[35] BLOOM, W.: Histopathology of Irradiation. National Nuclear Energy Series. New York, McGraw-Hill Book Company, 1948.